Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1. (original): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, said lyophilized bead comprising:

a thermally stable enzyme; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between about 53% and about 75% (w/w).

2. (original): The lyophilized bead of claim **1**, wherein said amplification occurs in a reaction mixture comprising a volume of between about 5 μL and about 200 μL.

3. (original): The lyophilized bead of claim **1**, further comprising a nucleoside triphosphate or a derivative thereof.

4. (original): The lyophilized bead of claim **1**, wherein said lyophilized bead has an average cross-section of between about 1 millimeter and about 4.5 millimeters.

5. (original): The lyophilized bead of claim **1**, wherein said weight percentage is between about 62% and about 75% (w/w).

6. (original): The lyophilized bead of claim **5**, wherein said weight percentage is between about 68% and about 75% (w/w).

7. (original): The lyophilized bead of claim **1**, wherein said thermally stable enzyme is selected from the group consisting of polymerase, ligase, and combinations thereof.

8. (currently amended): The lyophilized bead of claim **1**, further comprising a ~~hot-start methodology~~ component selected from the group consisting of an antibody that inactivates a polymerase and a wax or oil to sequester magnesium.

9. (original): The lyophilized bead of claim **1**, further comprising HEPES.

10. (original): The lyophilized bead of claim **1**, further comprising a probe.

11. (withdrawn): The lyophilized bead of claim **1**, further comprising a reverse transcriptase.

12. (original): The lyophilized bead of claim **1**, further comprising an internal control.

13. (withdrawn): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, said lyophilized bead comprising:

a forward polynucleotide primer;

a reverse polynucleotide primer; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between about 53% and about 75% (w/w).

14. (withdrawn): The lyophilized bead of claim **13**, wherein said amplification occurs in a reaction mixture comprising a volume of between about 5 μL and about 200 μL.

15. (withdrawn): The lyophilized bead of claim **13**, wherein said lyophilized bead has an average cross-section of between about 1 millimeter and about 4.5 millimeters.

16. (withdrawn): The lyophilized bead of claim **13**, wherein said weight percentage is between about 62% and about 75% (w/w).

17. (withdrawn): The lyophilized bead of claim **16**, wherein said weight percentage is between about 68% and about 75% (w/w).

18. (withdrawn): The lyophilized bead of claim **13**, further comprising HEPES.

19. (withdrawn): The lyophilized bead of claim **13**, further comprising a probe.

20. (withdrawn): The lyophilized bead of claim **13**, further comprising an internal control.

21. (withdrawn): The lyophilized bead of claim **13**, wherein said nucleic acid sequence is selected from the group consisting of bacterial, fungal, and viral nucleic acid sequences.

22. (withdrawn): The lyophilized bead of claim **21**, wherein said bacterial nucleic acid sequence is derived from a member selected from the group consisting of *Bacillus Anthracis, Yersinia pestis, Clostridium botulinum, Francisella tularensis*, Group B *Streptococcus*, *Neisseria gonorrhoeae, Chlamydia trachomatis,* and *Xylella fastidiosa.*

23. (withdrawn): The lyophilized bead of claim **21**, wherein said viral nucleic acid sequence is derived from a member selected from the group consisting of Vaccinia, West Nile Fever virus, Equine Encephalitis virus, and Foot and Mouth Disease virus.

24. (withdrawn): A method for the amplification of a nucleic acid sequence, said method comprising:

(a) dissolving a lyophilized bead in a liquid, wherein said lyophilized bead comprises:

a thermally stable enzyme; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between about 53% and about 75% (w/w), thus forming a reaction mixture; and

(b) subjecting said reaction mixture to an amplification reaction.

25. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a volume of between about 5 μL and about 200 μL.

26. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a nucleoside triphosphate or a derivative thereof.

27. (withdrawn): The method of claim **24**, wherein said thermally stable enzyme is selected from the group consisting of polymerase, ligase, and combinations thereof.

28. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a forward polynucleotide primer.

29. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a reverse polynucleotide primer.

30. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a probe.

31. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a nucleic acid comprising said nucleic acid sequence.

32. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises HEPES.

33. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises an internal control.

34. (withdrawn): The method of claim **24**, wherein said reaction mixture further comprises a hot start methodology.

35. (withdrawn): The method of claim **24**, wherein said lyophilized bead has an average cross-section of between about 1 millimeter and about 4.5 millimeters.

36. (withdrawn): A method for the amplification of a nucleic acid sequence, said method comprising:

(a) dissolving a lyophilized bead in a liquid, wherein said lyophilized bead comprises:

a forward polynucleotide primer;

a reverse polynucleotide primer; and

mannitol; and

wherein said lyophilized bead has a weight percentage of said mannitol of between about 53% and about 75% (w/w), thus forming a reaction mixture; and

(b) subjecting said reaction mixture to an amplification reaction.

37. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a volume of between about 5 μL and about 200 μL.

38. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a nucleoside triphosphate or a derivative thereof.

39. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a probe.

40. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a nucleic acid comprising said nucleic acid sequence.

41. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises HEPES.

42. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises a thermally stable enzyme.

43. (withdrawn): The method of claim **36**, wherein said reaction mixture further comprises an internal control.

44. (withdrawn): The method of claim **36**, wherein said lyophilized bead has an average cross-section of between about 1 millimeter and about 4.5 millimeters.

45. (original): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, prepared by a process comprising:

(a) creating an aqueous solution, said aqueous solution comprising:

a thermally stable enzyme; and

mannitol;

wherein said solution has a concentration of said mannitol between about 0.38 M (moles of mannitol/liter of solution) and about 0.99 M (moles of mannitol/liter of solution);

(b) quick-freezing the product of (a); and

(c) freeze-drying the product of (b).

46. (original): The lyophilized bead of claim **45**, wherein the product of (c) has an average cross-section of between about 1 millimeter and about 4.5 millimeters.

47. (original): The lyophilized bead of claim **45**, wherein the product of (c) further comprises a nucleoside triphosphate or a derivative thereof.

48. (original): The lyophilized bead of claim **45**, wherein said thermally stable enzyme is selected from the group consisting of polymerase, ligase, and combinations thereof.

49. (withdrawn): The lyophilized bead of claim **45**, wherein the product of (c) further comprises a reverse transcriptase.

50. (currently amended): The lyophilized bead of claim **45**, wherein the product of (c) further comprises a ~~hot start methodology~~ component selected from the group consisting of an antibody that inactivates a polymerase and a wax or oil to sequester magnesium.

51. (original): The lyophilized bead of claim **45**, wherein the product of (c) further comprises HEPES.

52. (original): The lyophilized bead of claim **45**, wherein the product of (c) further comprises a probe.

53. (original): The lyophilized bead of claim **45**, wherein the product of (c) further comprises an internal control.

54. (withdrawn): A lyophilized bead suitable for use in the amplification of a nucleic acid sequence, prepared by a process comprising:

(a) creating an aqueous solution, said aqueous solution comprising:

a forward polynucleotide primer;

a reverse polynucleotide primer; and

mannitol;

wherein said solution has a concentration of said mannitol between about 0.38 M (moles of mannitol/liter of solution) and about 0.99 M (moles of mannitol/liter of solution);

(b) quick-freezing the product of (a); and

(c) freeze-drying the product of (b).

55. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) has an average cross-section of between about 1 millimeter and about 4.5 millimeters.

56. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) further comprises a nucleoside triphosphate or a derivative thereof.

57. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) further comprises HEPES.

58. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) further comprises a probe.

59. (withdrawn): The lyophilized bead of claim **54**, wherein the product of (c) further comprises an internal control.

60. (withdrawn): A lyophilized bead suitable for use in microanalytic systems comprising:

a moisture-sensitive reactant; and

mannitol;

wherein said lyophilized bead has a weight percentage of said mannitol of between about 53% and about 75% (w/w); and

wherein said lyophilized bead has an average cross-section of between about 1 millimeter and about 4.5 millimeters.

61. (withdrawn): The lyophilized bead of claim **60**, wherein said weight percentage is between about 62% and about 75% (w/w).

62. (withdrawn): The lyophilized bead of claim **60**, wherein said weight percentage is between about 68% and about 75% (w/w).

REMARKS/ARGUMENTS

THE INVENTION

This invention is the discovery that mannitol when used as the major excipient can produce surprisingly smooth and uniform lyophilized beads for stably containing PCR reagents. This is contrasted with other carbohydrate excipients that do not produce such beads. Smooth and uniform beads is essential for commercial production of bead-based assay kits because such kits require the dispensing of large numbers of small quantities of beads in a highly reproducible manner.

STATUS OF THE CLAIMS

Claims 1-62 were pending. Claims 11, 13-44, 49 and 54-62 are withdrawn due to an election of Group 1. Claims 1-10, 12, 45-48 and 50-53 are pending. All the examined claims are rejected under §112 and/or §103.

CLAIM AMENDMENTS

Claims 8 and 50 have been amended to recite components expressly set forth in paragraph 26 of the specification.

RESTRICTION REQUIREMENT

Applicants acknowledge the withdrawal of the non-elected claims and the basis for the restriction. Applicants have not cancelled the non-elected claims at this time. Applicants wish to discuss the restriction requirement with the Examiner once allowable claims are identified, while reserving their right to petition for reconsideration of the restriction requirement.

OBJECTIONS

The specification was objected to for failure to present trademarked goods in proper form. As amended, the trademarks are capitalized and followed by a registration mark.

REJECTIONS

35 U.S.C. §112, 2nd paragraph

Claims 8 and 50 were rejected as indefinite for use of the term "hot start methodology". The primary concern was that the limitations of these two dependent, composition claims related to processes and not to physical elements of the claimed compositions. As amended, these two claims now recite physical components defining hot start methodology.

35 U.S.C. §103(a)

Park and Treml

The pending claims are rejected over Park and Treml. Park discloses mannitol as one of several polyols useful for stabilizing PCR reagents during lyophilization. Treml discloses forming lyophilized beads containing biological reagents by combining high molecular weight synthetic polymers with a carbohydrate. Nine carbohydrates are listed, but mannitol is not among them.

The Examiner combines Park and Treml to urge that the pending claims are obvious and unpatentable. Applicants acknowledge with appreciation the detailed explanation provided by the Examiner.

The Examiner, in recognition of the need to identify all the salient elements of the rejected claims, clearly understood that the applicants' discovery was that high amounts of mannitol provided a surprisingly superior bead. He goes on to state that Park does not teach high concentrations of mannitol but that Treml does disclose high levels of carbohydrate overlapping with applicants' claims and that mannitol is a carbohydrate.

Applicants respectfully request reconsideration in view of the following comments. As the Examiner knows, applicants' beads dramatically improved in appearance and uniformity when the mannitol levels increased above ~50%. This was unexpected, unpredictable and economically advantageous to those persons fashioning commercial quantities of PCR kits.

It is respectfully submitted that the Examiner has misunderstood the teachings of Treml with respect to the pending claims. The Examiner states that Treml does not disclose quantities of carbohydrates in terms of w/w but in terms of w/v. And that is the point where the Examiner's argument is flawed.

A closer reading of Treml will demonstrate that there is no suggestion to use the carbohydrate component as the major excipient in their lyophilized beads. The major excipient of Treml's beads is always the synthetic polymer. The carbohydrate is a minor component of the beads.

For a lyophilized product, the w/v of the original fluids used to construct the final dry product is irrelevant to the % w/w of the components of the final dry product. Let's look at this point in more detail.

The pending claims clearly dictate that more than 50% of the bulk or weight of the beads must be mannitol. To get this quantity of mannitol in the beads, the specification says to add solutions of mannitol at a percent of w/v that overlaps with the solutions used by Treml (column 5, lines 49-53). But this is where the overlap ends. Because the claimed beads are lyophilized beads, it is the w/w that defines the composition of the beads and not the starting solutions used to make the beads.

The w/w teachings of Treml are presented in the patent at column 5, lines 54-62. There it is clear that the synthetic polymer is the major component of the Treml dry beads. There is no teaching to suggest that the carbohydrate component should be present in greater than 50% of the total weight of the bead. The patent teaches:

> The percent solids in the reagent sphere is between 10% to 50%. Preferably, the percent solids are between 20% and 30%. The percent solids in the formulations described in the examples ranged from 21% for PCR reactions to 29% for DNA labelling reactions. The carbohydrates provide most of the mass in the

> formulations. Typically the buffer and enzymes account for 0.25-0.4 mg in any formulation. The carbohydrate polymer [high molecular weight synthetic weight polymer]accounted for 1.25-1.75 mg and melezitose accounts for 0.5-1 mg in any formulation described in the example.

Taking Treml's teaching in the worst light for the applicants and presuming that the beads are totally dry, the carbohydrate remains a minor component in their beads and is clearly outside of the claimed w/w range of greater than 53%. Using Treml's **lowest** figures for the PCR reagents and the polymer, you get .25 and 1.25 mg—which is a total of 1.5 mg. The **highest** level of carbohydrate is 1 mg and this gives us a % w/w of 1.0 mg carbohydrate to 2.5 mg total weight, or 40% w/w carbohydrate.

As the Examiner knows, the *prima facie* case of obviousness requires him to identify all the salient elements of the rejected claim in the prior art, a motivation to combine and a reasonable expectation that once combined the elements will function as expected. In our situation, neither Treml nor Park suggest adding enough mannitol to a lyophilized bead to render the mannitol the predominant solid component. And this is the critical discovery of this invention.

Having failed to identify overlapping w/w percentages in the prior art, there is a failure to identify a critical element of the claim and thus a failure of the *prima facie* case of obviousness. However, in an effort to be complete, applicants will briefly address the failure of the prior art to motivate the manufacture of a lyophilized bead with mannitol as the major excipient, and finally restate the surprising results that were observed with mannitol as the major excipient.

Motivation to combine is the second of the three elements of a *prima facie* case of obviousness. Motivation can be either express or implied. The Examiner's sole basis for motivation is the fact that mannitol is a carbohydrate that can stabilize enzyme activity (Park) and his erroneous belief that Treml's teachings of w/v led to the conclusion that their % w/w of carbohydrate overlapped with the claimed % w/w of greater than 53%. As explained above, Treml did not teach that carbohydrate should be present in greater amounts than the high

molecular weight synthetic weight polymer that forms the "glass-forming" filler material. In deed, the examples in Treml teach away from even the high ranges we presumed in our hypothetical.

In the Treml examples, the polymer Ficoll is at 12%, and the carbohydrate melezitose is at 5%. Even without formally calculating the relative % w/w, there is no way melezitose can be the major component of the Treml beads on a % w/w basis because it is lower in both molecular weight and percent than the synthetic polymer.

Finally, presuming an expectation that mannitol at any concentration could be expected to form bead shaped, lyophylized particles, there were the unexpected advantages of the claimed concentrations as clearly set forth in the specification and dramatically illustrated in Figure 1. The improved surface uniformity has great advantages in commercial production of bead based kits because the kits require dispensing of beads in highly reproducible amounts. Beads lacking uniformity of size and shape cannot be easily dispensed in a uniform and reproducible manner.

In view of the above remarks, applicants submit that the *prima facie* case of obviousness has been both rebutted by argument and traversed by evidence of surprising results. Reconsideration and withdrawal of the rejection of claims 1-8, 10, 12, 45-48, 50 and 52-53 over Park and Treml as obvious is requested.

Park, Treml and Kellogg

Claims 8 and 50 reciting hot start methods are rejected as obvious over Park, Treml and Kellogg. Park and Treml are relied upon as set forth above, and Kellogg is cited for disclosure of the polymerase antibody technique of hot starting PCR. In response, applicants rely on the arguments set forth above for claims 1-8, 10, 12, 45-48, 50 and 52-53. Claims 8 and 50 are dependent upon claim 1, and claim 1 is non-obvious for the reasons set forth above. Treml and Park fail to render PCR beads with mannitol as the major excipient obvious, and adding Kellogg disclosing hot start antibodies to the reference combination does not cure that failure.

Park Treml and Shively

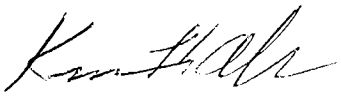
Claims 9 and 51 reciting buffering components of the PCR mix are rejected as obvious over Park, Treml and Shively. Park and Treml are relied upon as set forth above, and Shively is cited for disclosure of HEPES for use in PCR. In response, applicants rely on the arguments set forth above for claims 1-8, 10, 12, 45-48, 50 and 52-53. Claims 9 and 51 are dependent upon claim 1, and claim 1 is non-obvious for the reasons set forth above. Treml and Park fail to render PCR beads with mannitol as the major excipient obvious, and adding Shively disclosing HEPES to the reference combination does not cure that failure.

CONCLUSION

In view of the foregoing, applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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